deposition and a high expression of COL2A1, aggrecan and the a10 integrin sub-unit and low expression of COL1A2 and the a11 integrin sub-unit. After treatment at day 14 with 1 ng/ml IL-1b, we observed at day 15 up-regulations of 450-fold for MMP-1, 60-fold for MMP-13, 54-fold for ADAMTS-4 and MMP-3 and 10-fold for ADAMTS-5 and IL-1Ra. Down-regulations of 2.5-fold for COL2A1 and aggrecan were observed only at day 17. At the protein level a dose-dependent increase of total MMP-1 and MMP-13 was noted with less than 15% in the active form. The effect of a 1 day treatment with 1 ng/ml Il-1b (day14/15) disappears completely after 3 days (day 18).
In this culture model, in the absence of IL-1b, addition of ASU was without effect on the chondrocyte metabolism. However, treatment with ASU (10 ug/ml) from day 14 decreased significantly at day 16 the level of expression of MMP-3 and MMP-13 activated by a 1 day treatment (day 14/15) with 1 ng/ml IL-1b.
Conclusions: This in vitro model of chondrocyte culture in three dimensional (3D) seems well adapted to investigate the responses of these cells to inflammatory cytokines and to evaluate the potential anti-inflammatory effects of drugs. Under the conditions selected, chondrocytes maintained a cartilage phenotype and expressed a catabolic profile when stimulated by IL-1b. ASU were able to partially counteract this effect demonstrating their potential to reduce the deleterious effect of IL-1 reported in OA.

P209

EXPOSURE OF HUMAN CARTILAGE TISSUE TO LOW CONCENTRATIONS OF BLOOD FOR A SHORT PERIOD OF TIME LEADS TO PROLONGED CARTILAGE DAMAGE; AN *IN VITRO* STUDY

N.W. Jansen[1], G. Roosendaal[1], J.W. Bijlsma[1], J. DeGroot[2], F.P. Lafeber[1]
[1]UMC Utrecht, Utrecht, The Netherlands, [2]TNO, Leiden, The Netherlands

Purpose: Exposure of cartilage to blood as a consequence of joint bleeds leads to cartilage damage. In the present study we determined the thresholds for the exposure time and concentration that lead to prolonged cartilage damage caused by exposure of cartilage to blood. This information may be particularly useful in the discussion whether aspiration of blood from a joint after a haemarthros is indicated or not.
Methods: Healthy human articular cartilage tissue explants were cultured in the presence or absence of 50% v/v blood for 1, 2, 3 or 4 days or for four days in the presence of 0, 5, 10, 20, 30 or 50% v/v blood, both followed by a recovery period of 12 days. The effect of blood exposure on cartilage was determined by measuring matrix synthesis, release, and content, as well as MMP-activity.
Results: Exposure of cartilage to 50% v/v blood led to direct cartilage damage largely independent of the exposure time; a complete inhibition of matrix synthesis, an approximate doubling of the release, and increased MMP activity and a decreased proteoglycan content were observed. These effects persisted during recovery in the absence of blood but only after an initial exposure equal to or exceeding 2 days. Exposure of cartilage for 4 days to varying doses of blood led to concentration dependent cartilage damage. These effects were long lasting when the concentration equalled or exceeded 10% v/v blood. When a blood load of 10% v/v for 2 days was evaluated, the adverse effects on cartilage were also persisting. All data are statistically significant.
Conclusions: This study demonstrates that a 2-day exposure of cartilage to 10% v/v blood *(in vitro)* leads to prolonged impairment of joint cartilage. This suggests that aspiration of blood from a joint within 2 days should be considered in clinical practice to prevent blood induced joint damage.

P210

DEGENERATED CARTILAGE IS SLIGHTLY MORE PRONE TO BLOOD INDUCED CARTILAGE DAMAGE

N.W. Jansen, G. Roosendaal, J.W. Bijlsma, F.P. Lafeber
UMC Utrecht, Utrecht, The Netherlands

Purpose: Joint bleeds can occur after a joint trauma and are frequently seen in patients suffering from haemophilia. Even a limited number of these joint bleeds lead to severe joint destruction in later years. Currently the general opinion amongst physicians is that a few joint bleeds are acceptable. However, it could well be that degenerated cartilage that is impaired by previous joint bleeds or mechanical stress (e.g. osteoarthritic cartilage) is more susceptible to blood induced damage. When this holds true, this would implicate that specifically in case of degenerated cartilage, joint bleeds should be prevented to avoid joint destruction in later years. We therefore investigated whether degenerative and osteoarthritic cartilage are more susceptible to blood induced cartilage damage than healthy cartilage.
Methods: Healthy, degenerative and osteoarthritic human articular cartilage tissue explants were cultured in the presence or absence of 10% v/v blood for 2 days (a minimal blood load compared to a clinical joint haemorrhage), followed by a recovery period of 12 days. The effect of blood exposure to cartilage was determined by its effect on matrix synthesis, release, and content. Both the direct effects and the effects after the recovery period were determined.
Results: When healthy cartilage was exposed to blood, this resulted in a direct decrease of the matrix synthesis (-73%), and an increase in release of matrix components (+148%). These effects were still present after the recovery period and at that time proteoglycan content of the matrix was decreased (-18%). The degenerative and osteoarthritic cartilage had the characteristic decreased synthesis, increased release, and decreased content of proteoglycans. Release of proteoglycans after the recovery period was slightly but statistically significantly elevated for the degenerated cartilage compared to healthy cartilage ($p<0.05$). The other parameters were not significantly changed in degenerated cartilage compared to healthy cartilage.
Conclusions: This study demonstrates that degenerated and osteoarthritic cartilage are slightly more susceptible to the adverse effects of blood exposure than healthy cartilage. It might therefore be of importance to prevent joint bleeds in patients with degenerated cartilage due to e.g. osteoarthritis. Moreover, aspiration of blood in case of a joint haemorrhage is indicated under these conditions.

P211

A ROLE FOR APOPTOSIS IN THE ETIOPATHOGENESIS OF OSTEOCHONDRITIS DISSECANS OF THE ANKLE CARTILAGE

B. Grigolo[1], L. De Franceschi[1], G. Lisignoli[1], L. Roseti[1], R. Buda[1], F. Vannini[1], S. Giannini[1], A. Facchini[2]
[1]Istituti Ortopedici Rizzoli, Bologna, Italy, [2]Università degli Studi di Bologna, Bologna, Italy

Purpose: Osteochondritis dissecans (OCD) is a common disease with unknown aetiology which particularly affects young people. It is characterized by a separation of a cartilage fragment with the subchondral bone. Many hypotheses about its aetiopathogenesis have been proposed, one of the most considerable, the start of a catabolic program following an initial traumatic event. The aim of the study was to highlight these mechanisms and to look for an apoptosis activity as one of a probable cause of the pathology development.

Methods: Human articular cartilage was obtained from ankle joints of patients who had undergone arthroscopy for autologous chondrocytes transplantation and from multiorgan donors (NC). Cartilage samples were analyzed histologically, immunohistochemically and by in situ cell death detection methods.
Results: Apoptosis activity was visualized in all the OCD samples and was particular evident in the superficial layer. The same cells stain positive for anti-nitrotyrosine antibodies. The morphological appearance of the detached cartilaginous fragments was comparable to normal hyaline cartilage.
Conclusions: The presence of apoptotic cells in the superficial layers of articular cartilage of OCD patients gives further evidences about the role of catabolic pathways in the development of this pathology. This finding could identifies a novel therapy for its treatment based on a pharmacological inhibition by different substances as cytokines and growth factors able to limit the action of some molecules involved in the apoptosis cascade.

P212

DIFFERENCE IN THE CHARACTERRISTICS BETWEEN ARTICULAR CHONDROCYTES AND COSTAL CHONDROCYTES UNDER CULTURE CONDITION

M. Katouda, T. Soejima, T. Inoue, K. Tabuchi, H. Murakami, T. Kanazawa, K. Noguchi, K. Nagata
Kurume University School of Medicine, Kurume, Japan

Purpose: It is well established that injuries involving the articular cartilage surface which are confined to the cartilage tend to undergo little restoration because cartilage has only a slight self-healing capacity. In 1994 Britberg et al.reported on the clinical resuts of the transplantation of human autologous chondrocytes in a monolayer system. On the other hand Popko (Folia Morphol.2003)reported a rabbit model of articular cartilage repair using cultured costal chondrocytes. Based on those finding costal chondrocytes thus appear to be a potentially useful transplant source for the cartilage repair. However, there have so far been few reports comparing costal chondrocytes with articular chondrocytes at the cell level in detail. We therefore reviewed the differences between articular chondrocytes and costal chondrocytes under various kinds of culture conditions.
Methods: Both articular cartilage specimens and costal cartilage specimens were obtained from young Japanese white rabbits. Next, the chondrocytes derived from each cartilage specimen were isolated and maintained in a monolayerculture until reaching the third passage. Thereafter, each chondrocyte specimen was shifted to a 3-D culture of collagen gels and then were maintained for 3 weeks.The cell proliferation kinetics, the expression of collagens, and the expression of proteoglycan were analyzed by immunohistochemical stainings a cell proliferation assay, and realtime-PCR throughout the all of the passages. The results were then compared between the articular chondrocytes and costal chondrocytes.
Results: The costal chondrocytes were found to be similar to the articular chondrocytes through each passage of monolayer culture in the cell proliferation assay, according to immunohistochemical staining and realtime-PCR for collagen expression, and also based on the findings of Alucian-Blue staining for proteogrican expression. In the case of the 3-D culture,however, articular chondrocytes were found to be stronger appearance than costal chondrocytes according to immunohistochemical staining and realtime-PCR for collagen expression, and safranin-O staining for proteogrican expression
Conclusions: Under these culture conditions, both chondrocytes underwent dedifferentiation once in monolayer-culture. In addition they again underwent differentiation after being subjected to a 3-D culture. We followed the same course. On the other hand, the costal chondrocytes demonstrated a weaker ability to produce matrices than articular chondrocytes. Kitaoka et al (J cell Biochem, 2001) reported that articular chondrocytes and costal chondrocytes showed a similar phenotype regarding the chondrocyte matrix. In addition, Sato et al. transplanted a costal cartilage in animal experiments in a knee cartilage defect and reported good cartilage reproduction. These reports suggest the likelihood that costal chondrocytes can be transplanted into defect of articular cartilage.
Based on the above findings, costal cartilage cells are therefore considered to be very useful based on the following two points: It represents the largest amount of permanent cartilage in the human body and the required surgical procedure is easy to perform, thereby causing less damage to the donor site. We herein described a method to improve the culture condition when using costal cartilage for transplantation, however, futher study is necessary before clinical trials can be started.

P213

TGF-β1, ALONG WITH A SWITCHING-OFF PP2A ACTIVITY, PROTECTS NORMAL HUMAN CHONDROCYTES FROM Ro 31-8220 AND TNF-α INDUCED APOPTOSIS

M. Lires-Deán, M.J. López-Armada, B. Caramés, B. Cillero-Pastor, B. Lema, F.J. Blanco
Osteoarticular and Aging Research Laboratory. Biomedical Research Center, CHU Juan Canalejo, A Coruña, Spain

Purpose: In this work, we studied if TGF-β1 is able to protect normal human chondrocytes from apoptosis induced by an *in vitro* model (TNF-α + Ro), as PP2A is, at least partially, switched-off.
Methods: Human normal cartilage was obtained from the femoral heads of 8 patients each. Cartilage was obtained from cadavers who had no history of joint disease and who had macroscopically normal cartilage. PP2A activity was estimated by measuring the absorbance of a molybdate:malachite green:phosphate reaction complex. Apoptosis was asessed by ELISA cell death, and with the fluorescent stain DAPI (4',6-diamidino-2-phenylindole, dihydrochloride).
Results: It was established two groups of cells, one group was preincubated for 120h only with TGF-β1, while the another group was incubated with TGF-β1 plus PP2A Inhibitor Protein. Afterwards, both groups were stimulated with TNF- α and Ro for 16h. First of all, we show that TGF-β1 stimulate PP2A activity (TGF-β1 156% vs Basal 100%; $p<0.05$).Preincubation of TGF-β1 plus PP2A Inhibitor Protein reduced internucleosomal DNA breakage as compared with TGF-β1 only (TGF-β1 plus PP2A Inhibitor Protein + TNF-α+Ro 59.0% vs TGF-β1 + TNF-α+Ro 100%; $p<0.05$). Furthermore, nuclear morphology typical of apoptosis was more widespread in the group of cells with only TGF-β1. As complementary control, apoptosis on chondrocytes only with PP2A Inhibitor Protein was also assesed; results did not show protection.
Conclusions: These results show the major role that PP2A plays in the outcome of TGF-β1 signal transduction, giving the potential of modulate TGF-β1 pathway, by manipulating the degree of PP2A activity, to produce a particular desired therapeutic outcome.